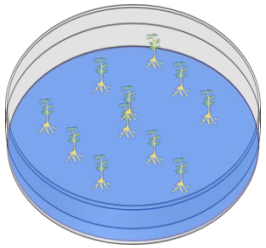
Cairo University
Faculty of Pharmacy

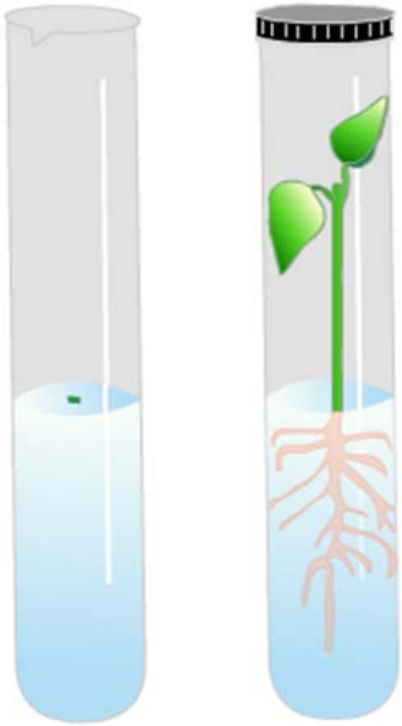
PLANT CELL
&
TISSUE CULTURE

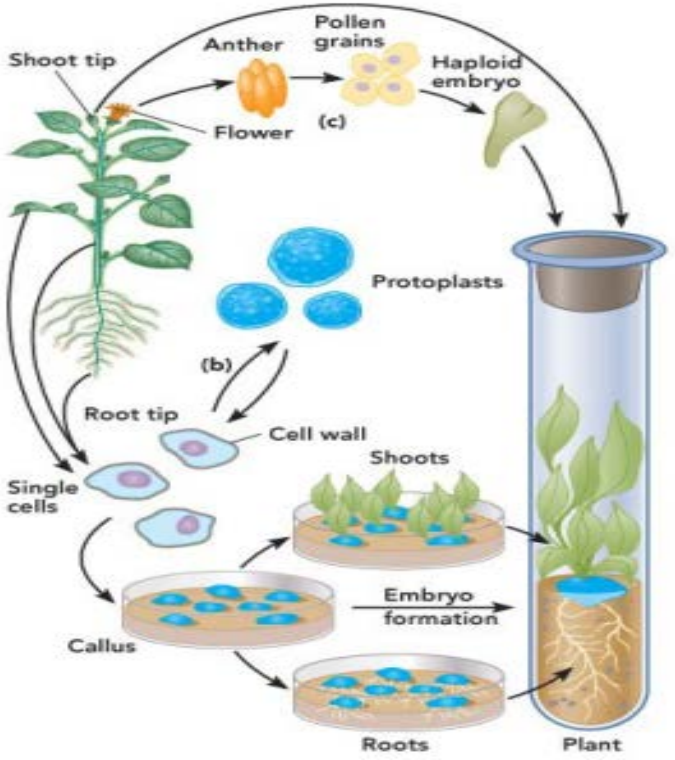


DR. OMAR SABRY

PLANT CELL & TISSUE CULTURE

- In vitro aseptic cultivation of Isolated plant organs or cells (root tips, shoot tips, leaves, embryos , cells on a nutrient medium).

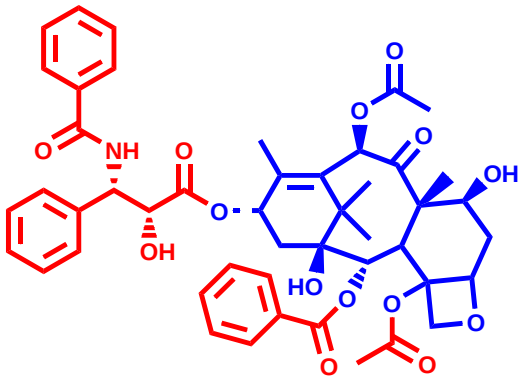


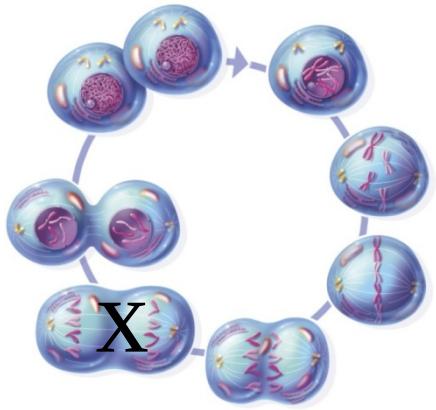


PLANT CELL & TISSUE CULTURE

- **Development of commercial production of expensive bio-medicaments (Taxol).**

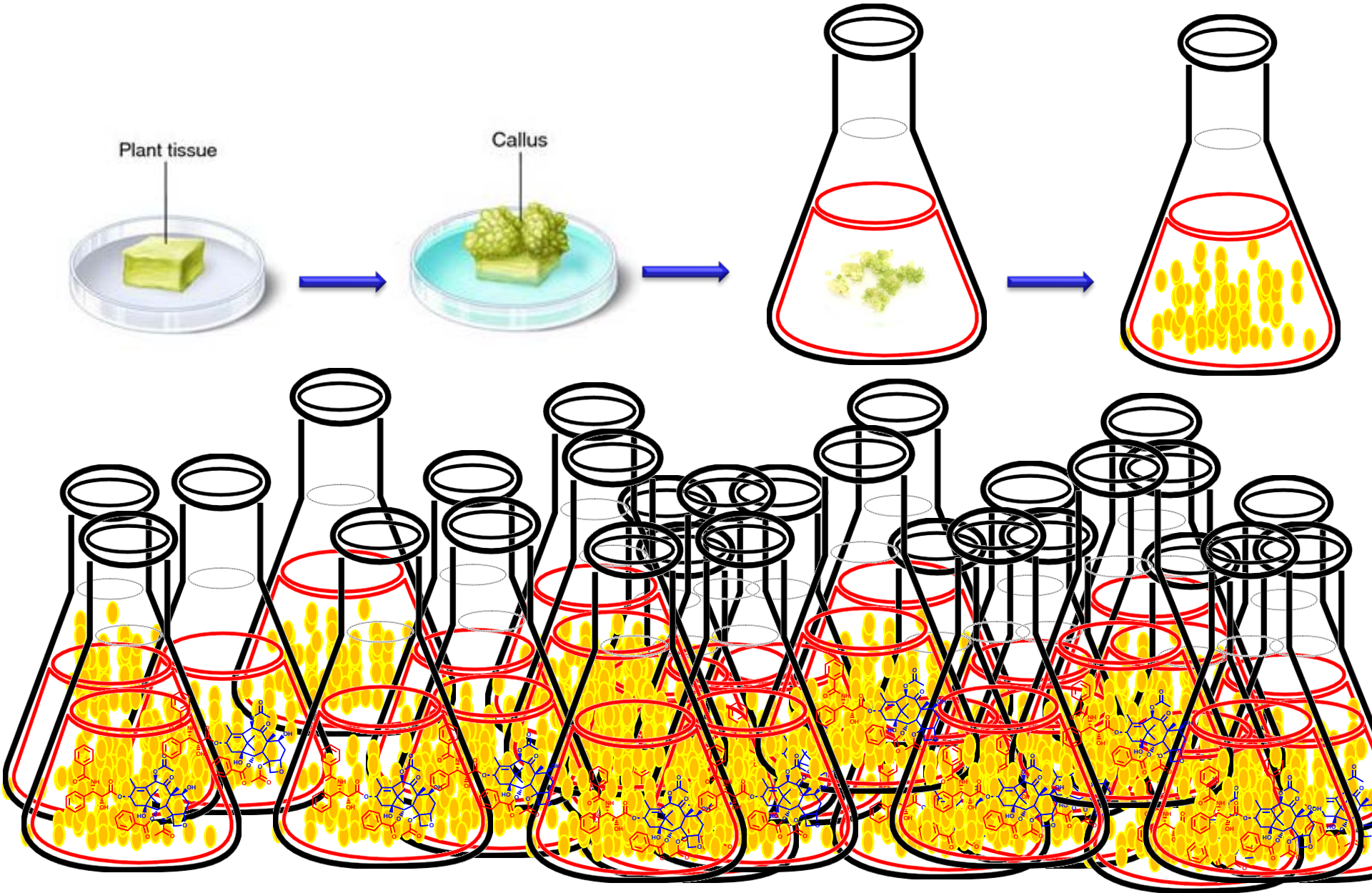
- **Anti-cancer from *Taxus brevifolia*.**





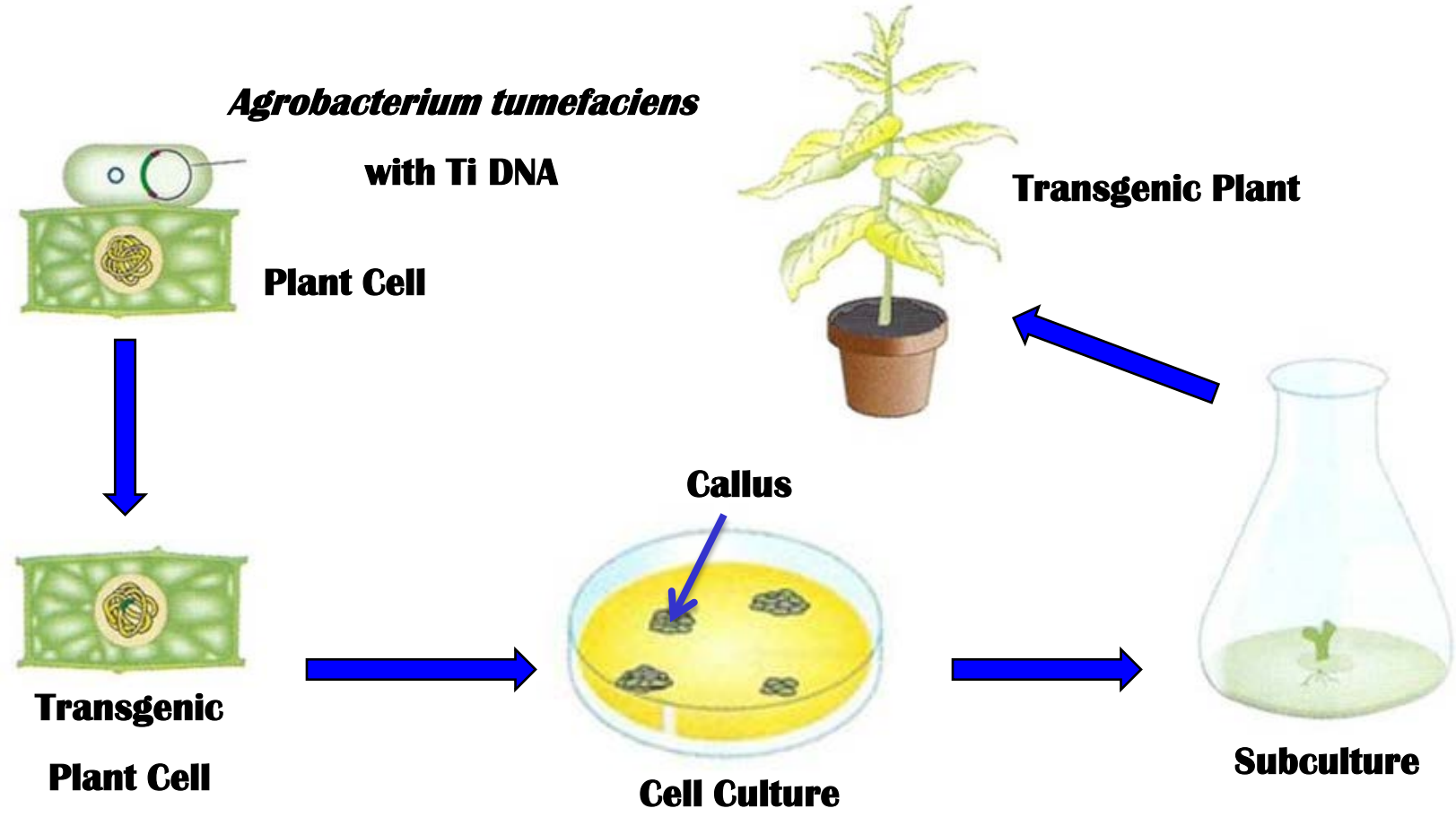
- **100 years old tree suitable for stem bark**
- **3 mature trees = 1 gm of Taxol.**
- **2 gm Taxol for Cancer treatment.**

PLANT CELL & TISSUE CULTURE



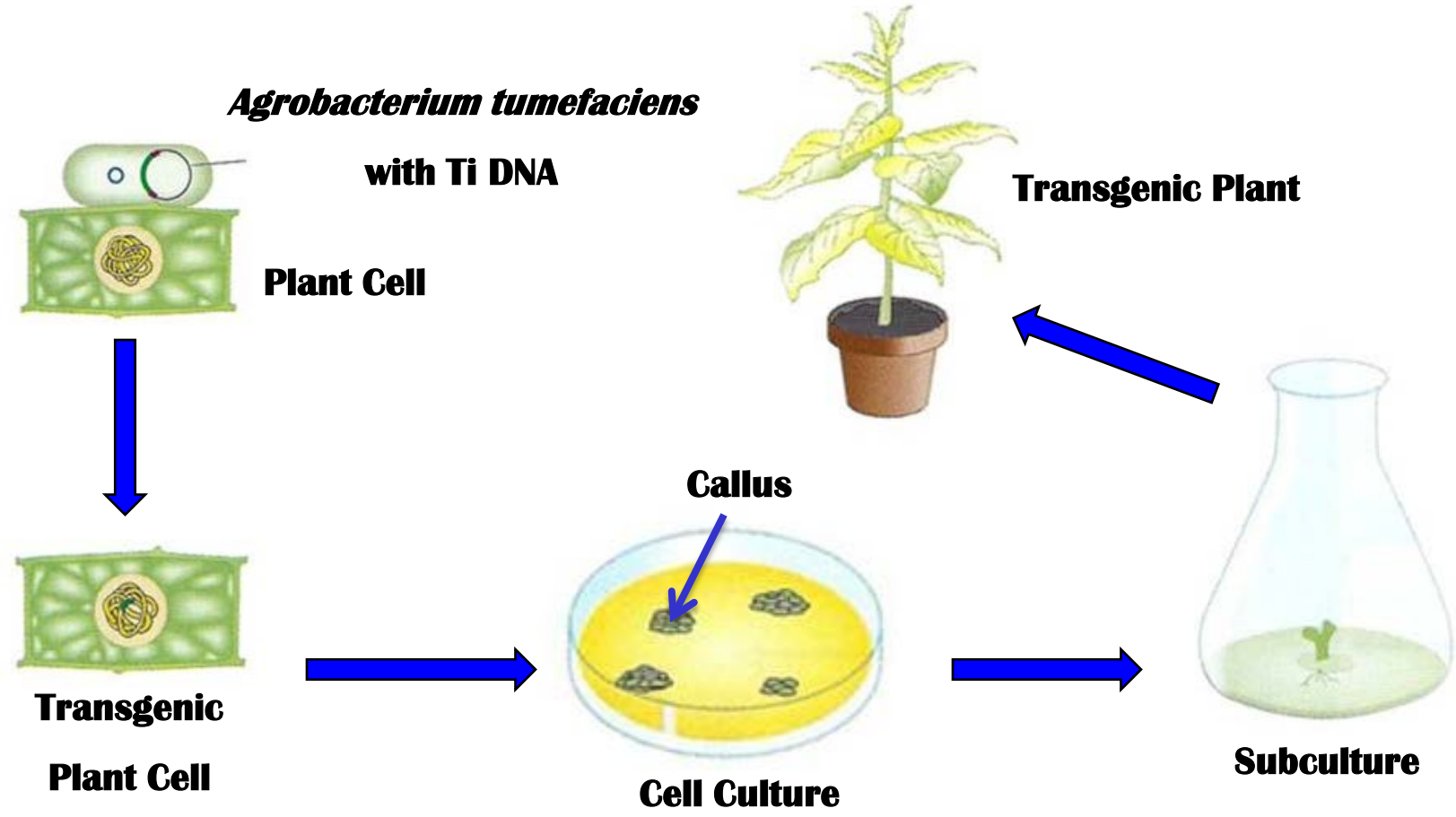
PLANT CELL & TISSUE CULTURE

- **Improvement of medicinal plants by genetic engineering e.g. *Atropa belladonna* gives atropine and morphine.**



PLANT CELL & TISSUE CULTURE

- **Improvement of medicinal plants by genetic engineering e.g. Insects resistant and microbes resistant plants.**



PLANT CELL AND TISSUE CULTURE LABORATORY

(A) Washing area

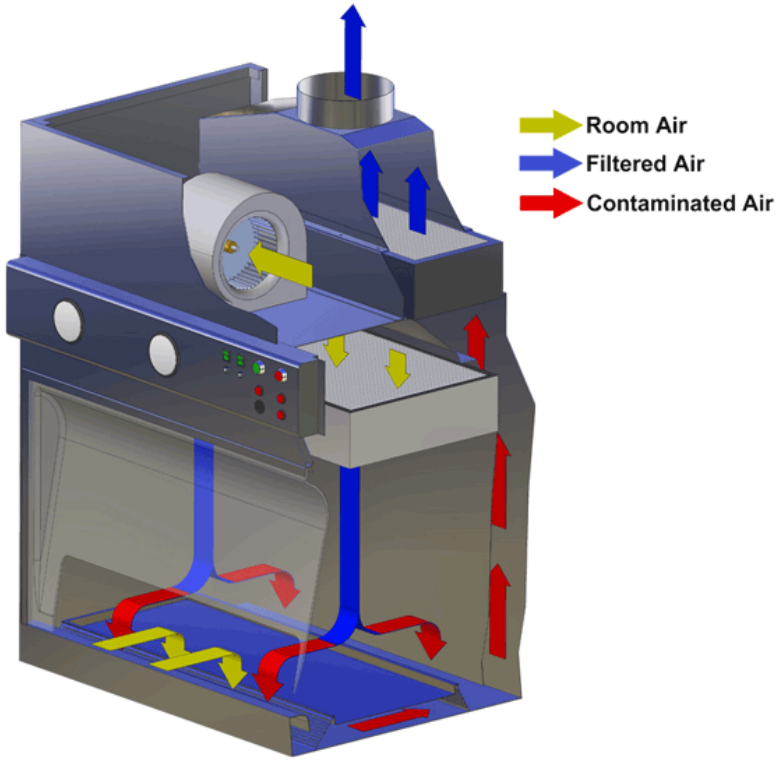
PLANT CELL AND TISSUE CULTURE LABORATORY

(B) Media preparation room

PLANT CELL AND TISSUE CULTURE LABORATORY

(C) Transfer Area

Laminar air - flow cabinets

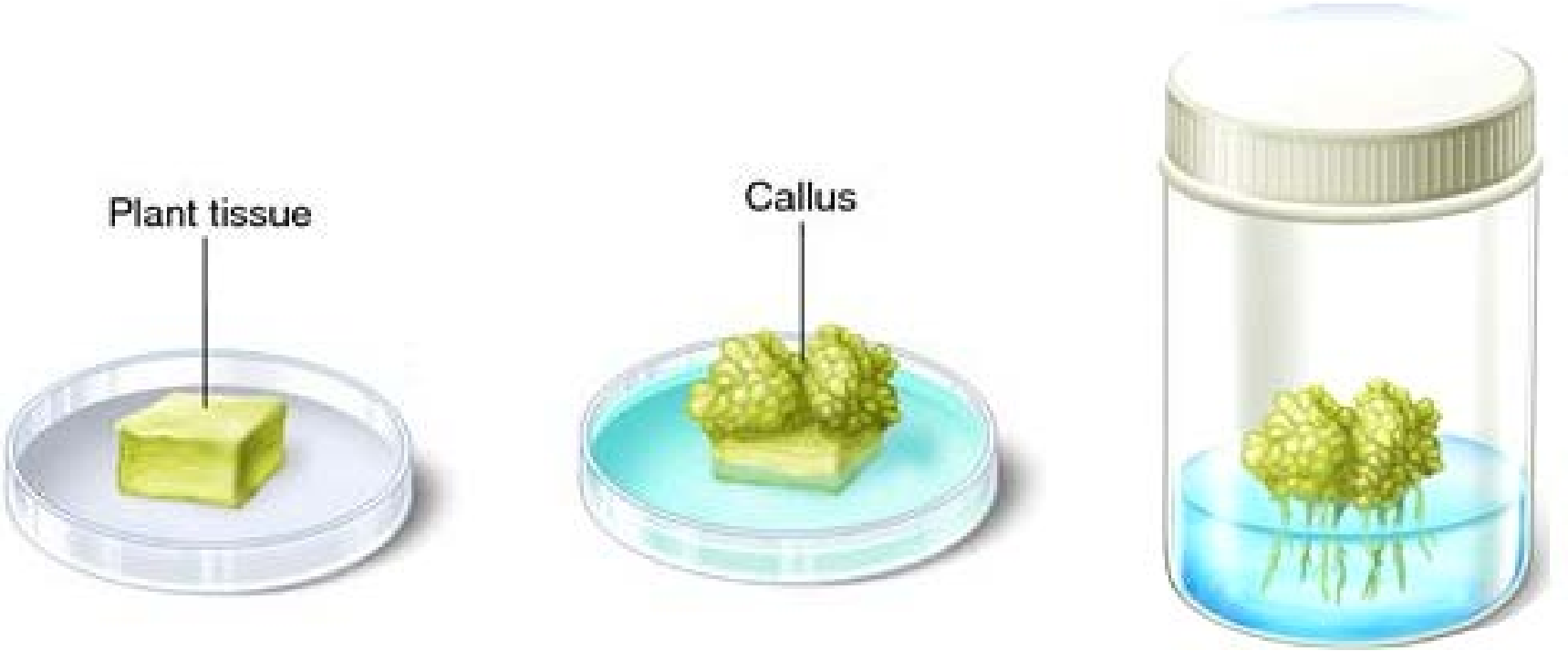


PLANT CELL AND TISSUE CULTURE LABORATORY

(D) Culture room

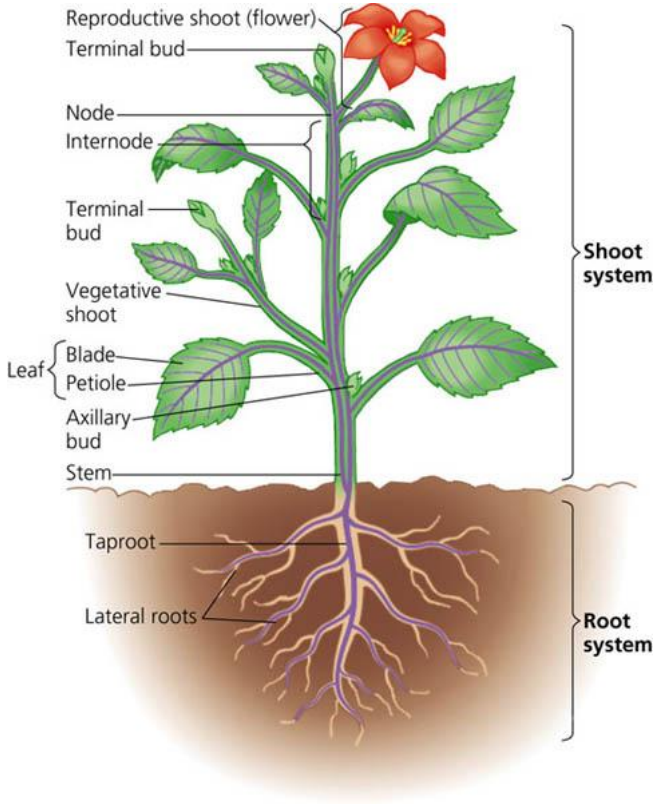
CULTURE VESSELS

- Borosilicate or Pyrex glassware should be used.
- Soda glass toxic to tissues with repeated use.

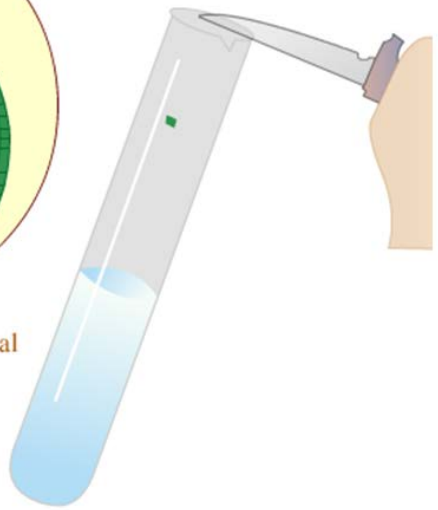


SOURCES OF TISSUE EXPLANTS

- **Organ with meri-stimatic cells Pith, root tips, shoot tips, embryos, anthers and young leaves.**

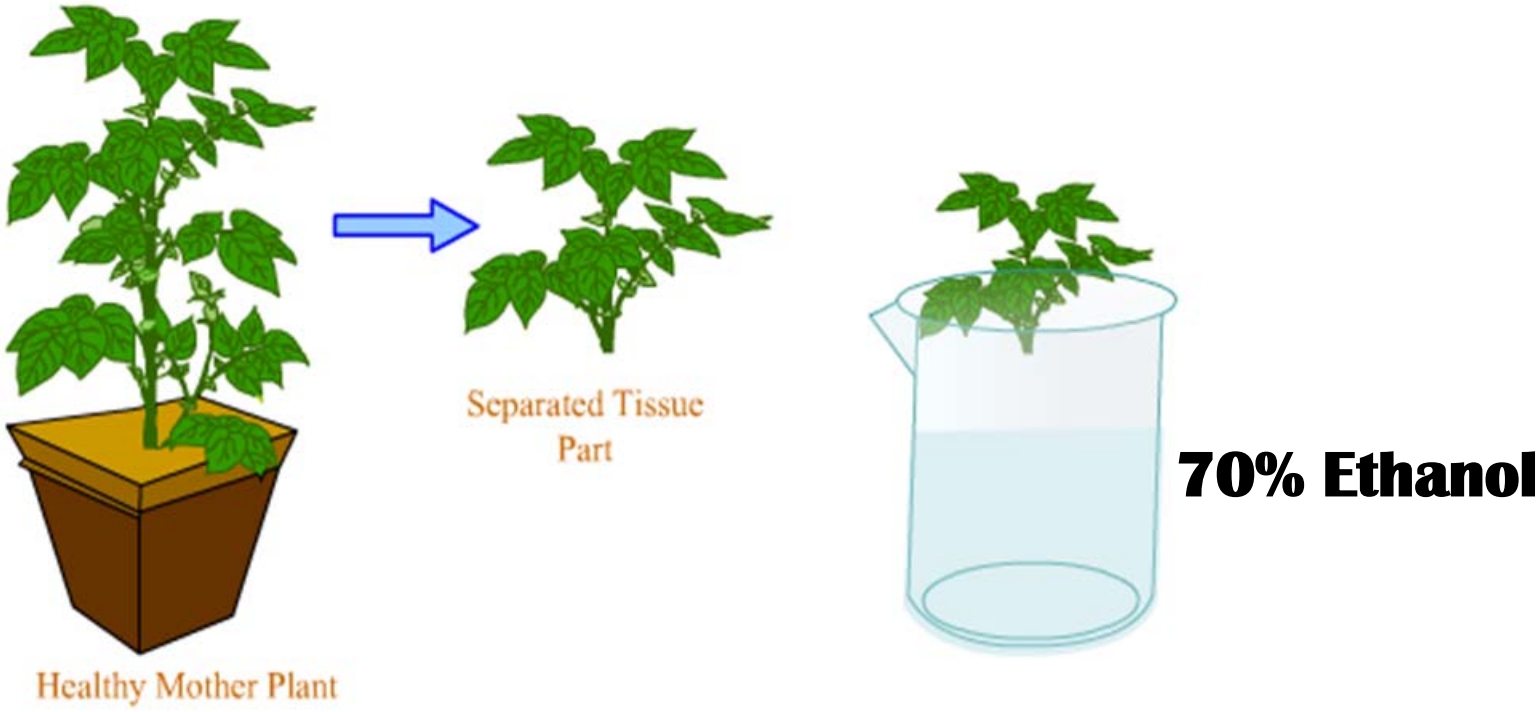


Meristematic tissue of terminal (microscopic appearance)



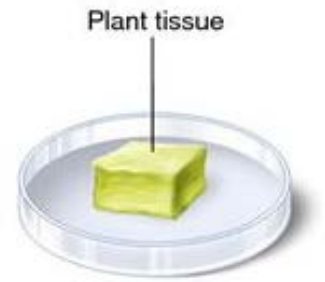
STERILIZATION OF PLANT MATERIAL

- **Tissues must be surface sterilized before planting on the nutrient medium.**

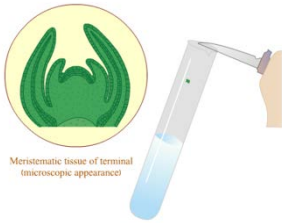


STERILIZING AGENTS USED IN PLANT STERILIZATION

- 70% ethyl alcohol (No methanol)
- Sodium hypochlorite 25%

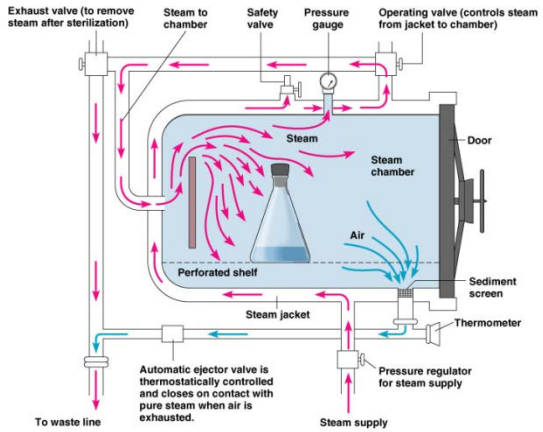


- After disinfection, carefully trim by cutting away all bleached and dead tissue .
- Rectangular or cylindrical pieces approximately 5 mm in diameter excised transferred to agar plates containing nutrient medium.



STERILIZATION OF GLASS WARE, PLASTIC WARE AND OTHER INSTRUMENTS

- **Autoclaving or dry heating in an oven at 160 -180°C for 3hr.**

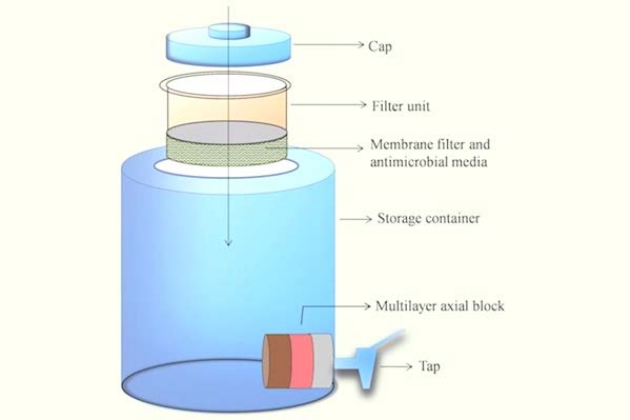


- **Plastic ware can be heat sterilized by autoclaving at 121 °C.**

- **Forceps, scalpels, needles and spatulae sterilized by 70% or 95 % ethanol followed by flaming and cooling.**

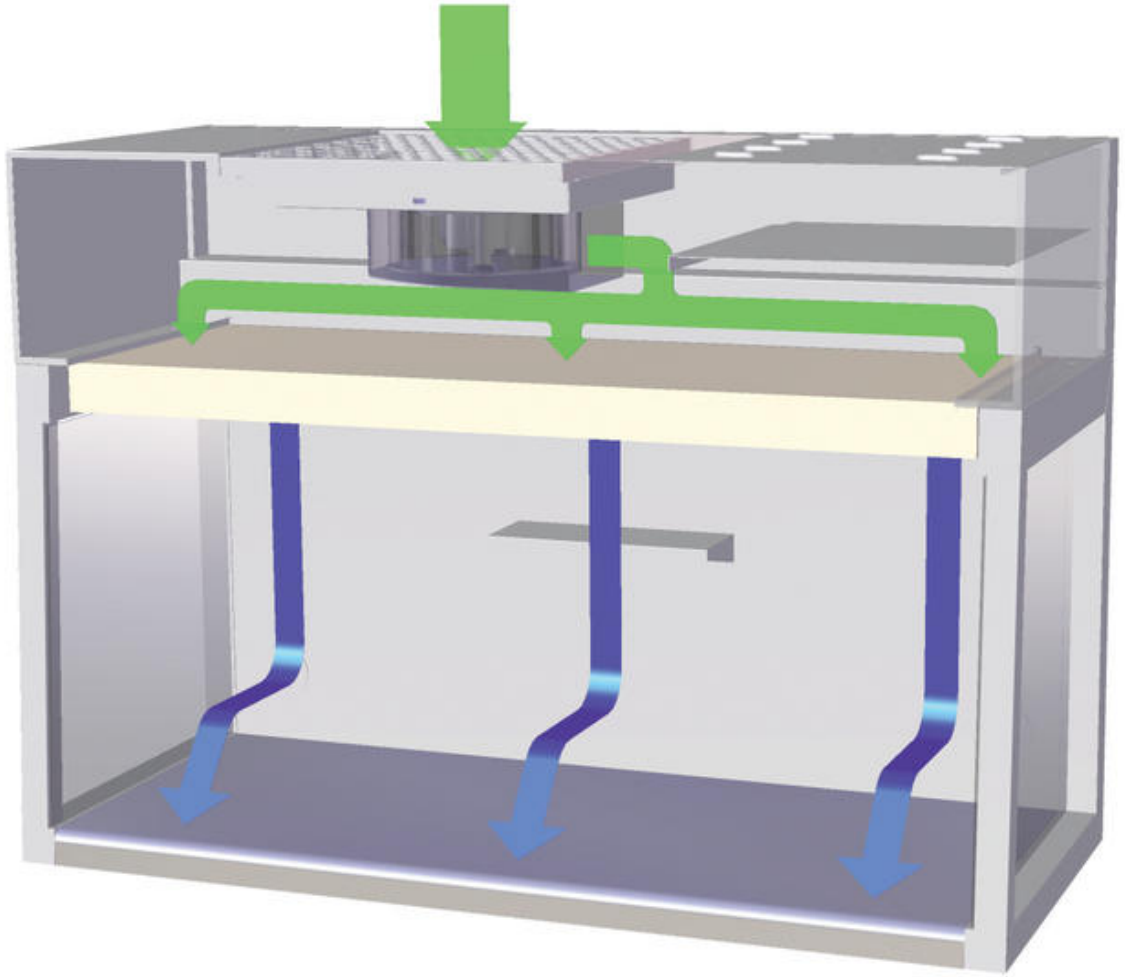
STERILIZATION OF MEDIA

- Some growth factors are thermolabile (No autoclaving).
- Medium minus the heatlabile compound autoclaved in a flask kept in the sterilized hood to cool down.
- Solution of the thermolabile compound is sterilized by Micropore membrane filter.



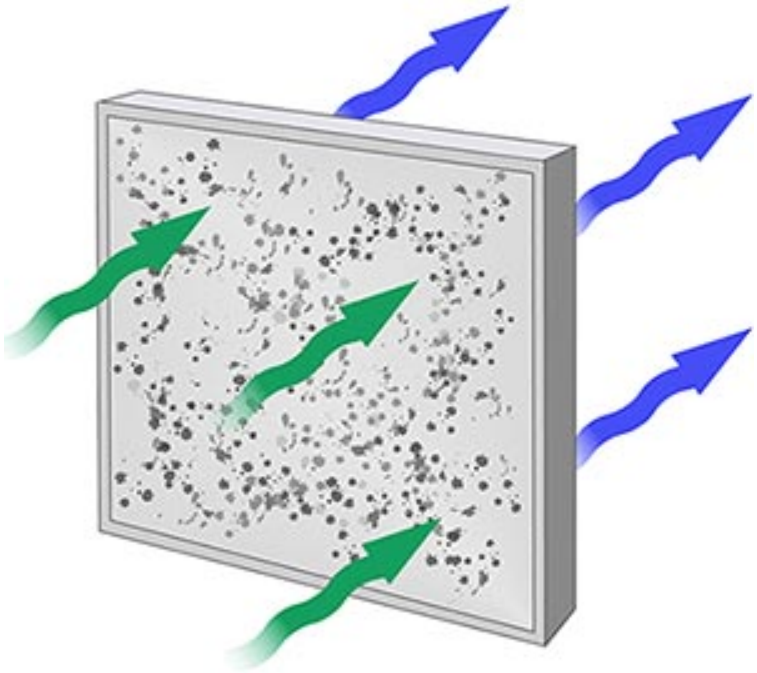
STERILIZATION OF TRANSFER AREA

- **Laminar airflow cabinets**



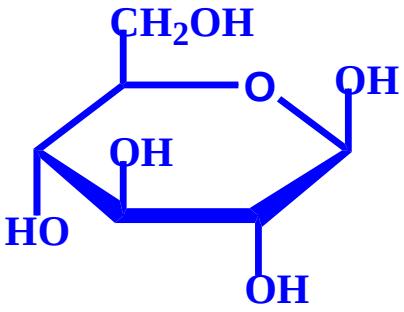
STERILIZATION OF AIR

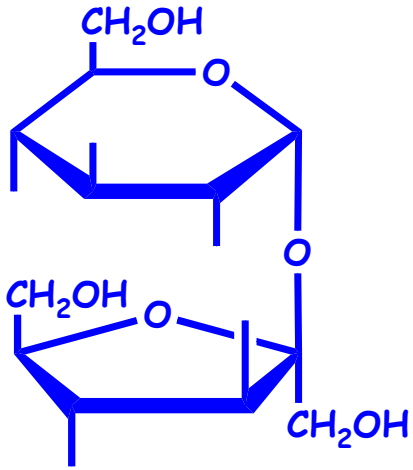
- **High efficiency particulate air (HEPA) filter**

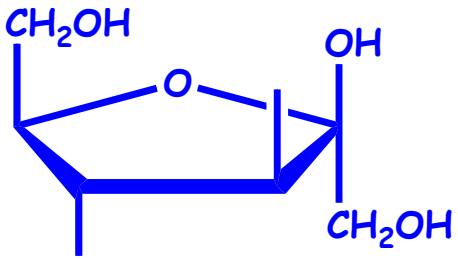


MEDIA CONSTITUENTS

- **Inorganic Nutrients**
  - Macro - or major elements (6): e.g. Nitrogen – Calcium.
  - Micro - or minor elements (6): e.g. Iron - Cu





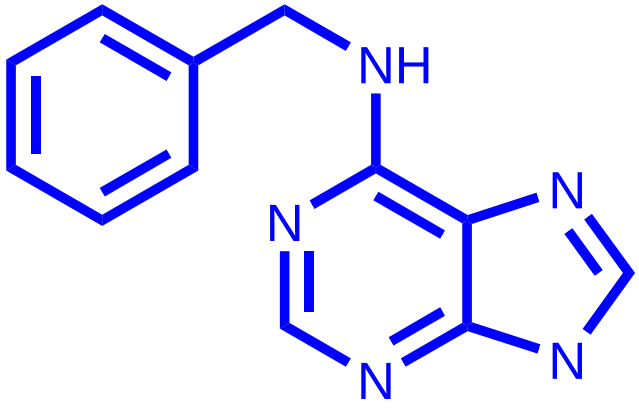


- **Organic Nutrients**
  - Nitrogenous substances e.g. Vitamins. Thiamine - Pyrodoxol
  - Carbon Source e.g. **Sucrose 2-5%, glucose and fructose**

GROWTH HORMONES

CYTOKININS

- BAP (benzyl amino purine)



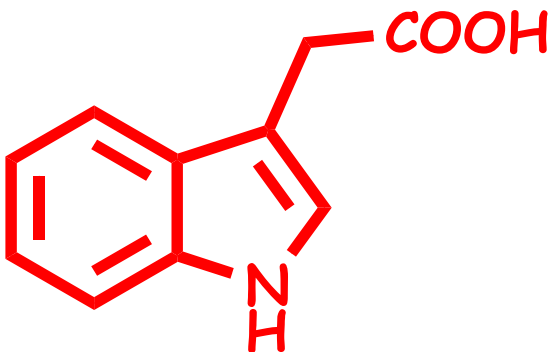
- Shoots emerge, Plant rooted with auxin, transferred to potting soil for further growth in the greenhouse as normal plants.

- Equal concentrations gives unorganized fashion

GROWTH HORMONES

AUXINS

- IAA (Indole acetic acid)



- Excess of auxins promotes growth of roots

- Excess of cytokinins yield shoots.

AGAR

- Generally used at a concentration of 0.8 - 1.0%.
- Higher concentration the medium becomes hard not allow the diffusion of nutrients into the tissues.

- Not an essential components of the nutrient medium.

CELL SUSPENSION CULTURE

- **Single cells and aggregates can be grown as suspension cultures in liquid medium.**

CELL SUSPENSION CULTURE

- Regularly aerated either by bubbling sterile air, or gentle agitation.
- Liquid medium may prove superior to agarified medium.
- pH of the medium 5.0 - 6.0 before sterilization.

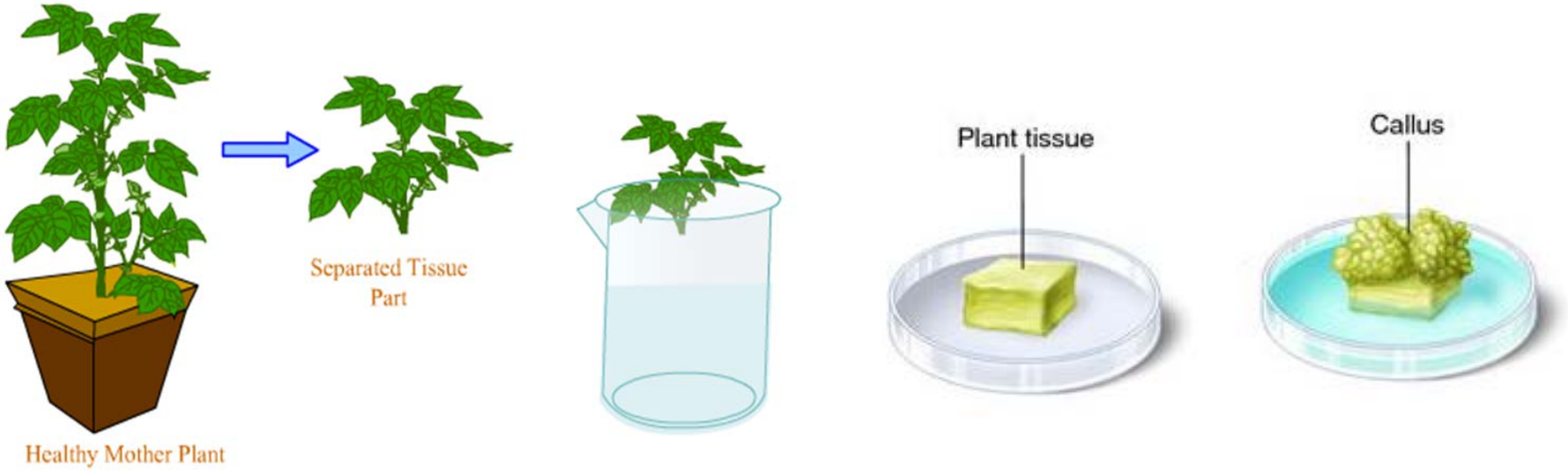
MEDIA SELECTION

- Start with known basal medium.
- Minor qualitative and quantitative changes, through series of experiments a new medium may be evolved.
- Simplest method commercially available dry powdered media, containing inorganic salts, vitamins and amino acids.

STAGES OF CULTURING

1- PREPARATION OF THE EXPLANT

- **Piece of stem, leaf, or root is disinfected with alcohol or sodium hypochlorite solution and cultured in a specific culture medium.**



STAGES OF CULTURING

2- MULTIPLICATION

- Number of cells increases.
- Cells cultured from a callus in the presence of low concentration of auxin and cytokinin.
- Calli placed in liquid medium and grown in suspension or placed on a solid substrate to be cultured.

STAGES OF CULTURING

- Callus cultures subdivided and re-cultured .
- Exposed to growth hormones, Calli form shoots and roots.
- Callus formation and maintenance must be taken in consideration because when cultured for several weeks any callus show signs of aging.

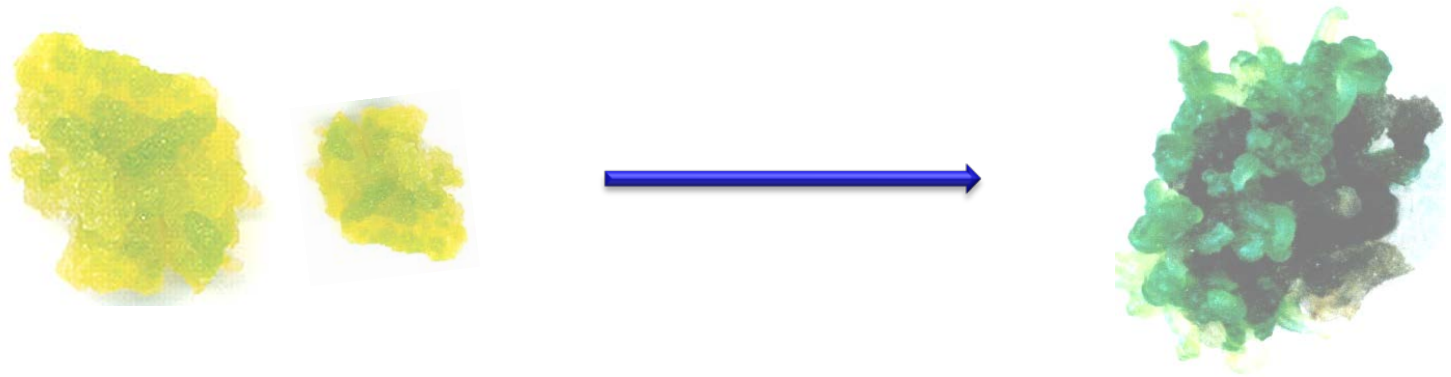
STAGES OF CULTURING

3. PRE-TRANSPLANT STAGE

- Allows the formation of roots, shoot development, maturation, and development.

- Cytokinins are reduced or eliminated and auxins are added to promote root development.

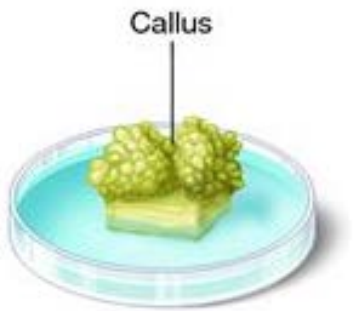
FACTORS INDUCING AGING



- Exhaustion of the nutrients.
- Inhibition of nutrient diffusion.
- Accumulation of toxic metabolites.
- Callus tissues periodically transferred to fresh medium (sub-culture) at intervals of 4-6 weeks.

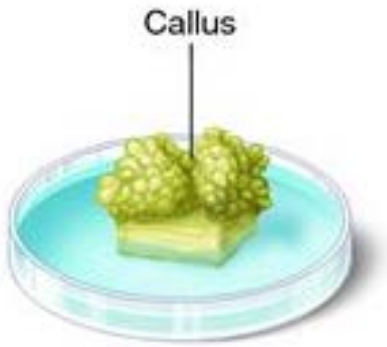
PREPARATION OF CELL SUSPENSION CULTURES

- Sections from surface – sterilized plant organs on a nutrient medium containing suitable hormones (auxins and cytokinins).
- The explant exhibits callusing.
- Callus separated from the parent explant transferred to fresh medium.

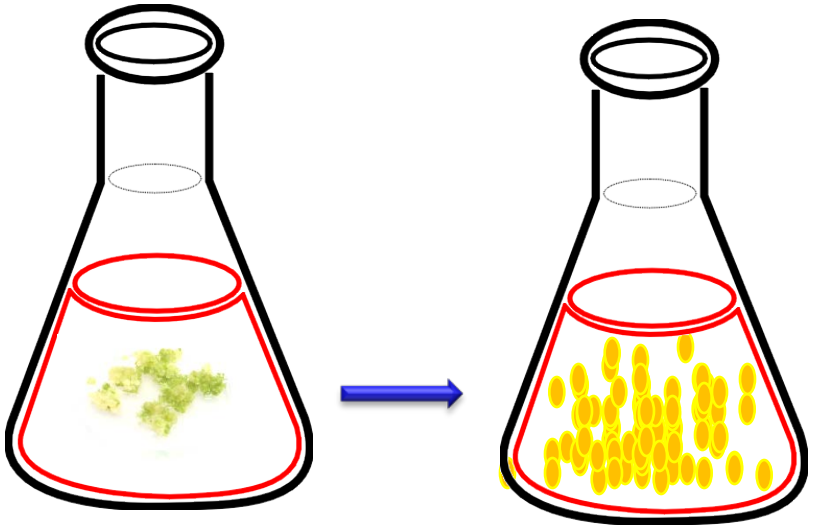


PREPARATION OF CELL SUSPENSION CULTURES

- **Repeated sub-culture on the agar improves friability desirable for raising a fine cell suspension in liquid medium.**

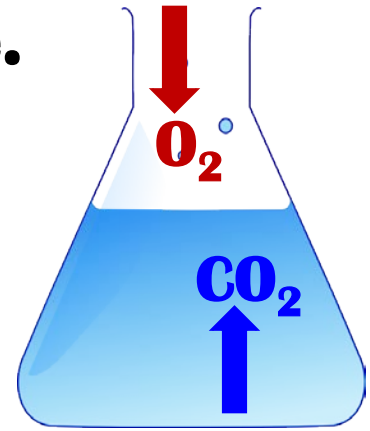


- **On transfer to liquid medium gave fine suspension.**



AGITATION FUNCTIONS

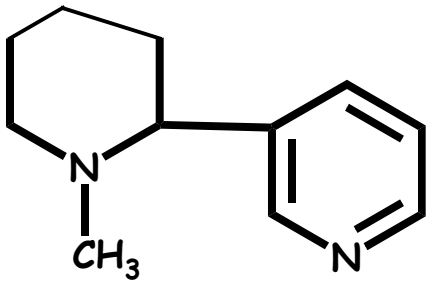
- ❑ **Breaking clumps into single cells.**
- ❑ **Uniform distribution of cells in the medium.**
- ❑ **Gaseous exchange.**

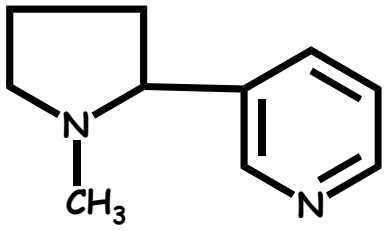


PLANT CELL & TISSUE CULTURE

Why some cell cultures may not produce the natural compounds or produce them in very small amounts?

- Biosynthesis by cooperation of different organs
- Lysine amino acid is converted to anabasine in tobacco roots
- followed by the conversion of anabasine to nicotine in leaves.



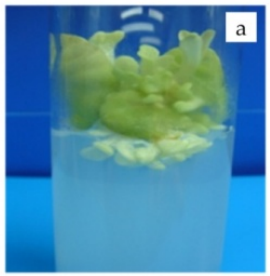


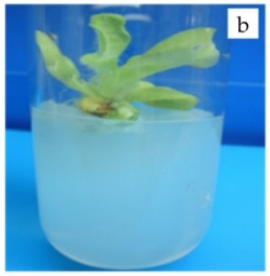
Nicotine

PLANT CELL & TISSUE CULTURE

➢ **Callus and shoot cultures of tobacco can produce only trace amounts of nicotine because they lack anabasine.**

Tissue cultured Tobacco

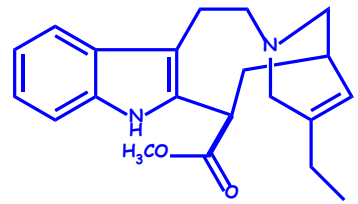




❑ **Compound may be produced in only one organ, or in one cell.**

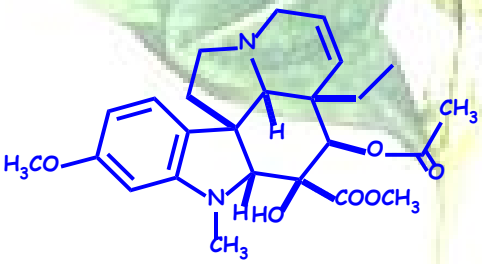
PLANT CELL & TISSUE CULTURE

- Some degree of differentiation in a cell culture of *Catharanthus roseus* must occur before vincristine can be biosynthesized.



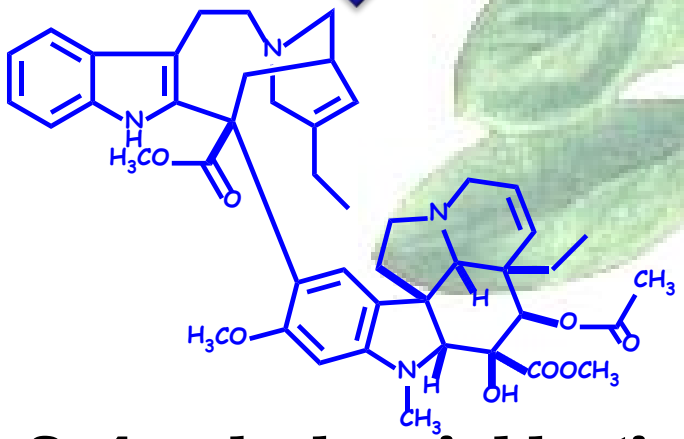
Catharanthine

+



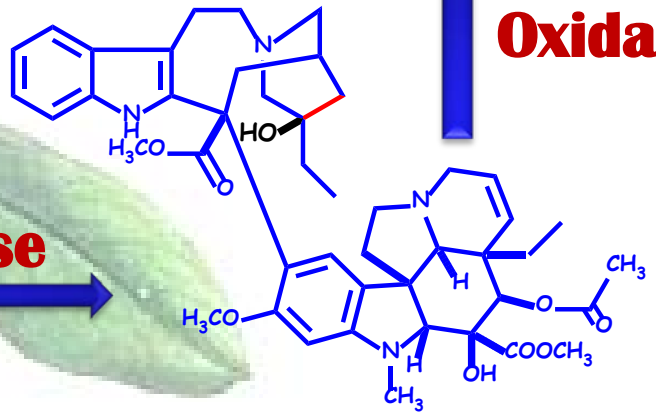
Vindoline

3-,4- anhydro Vinblastine Synthase



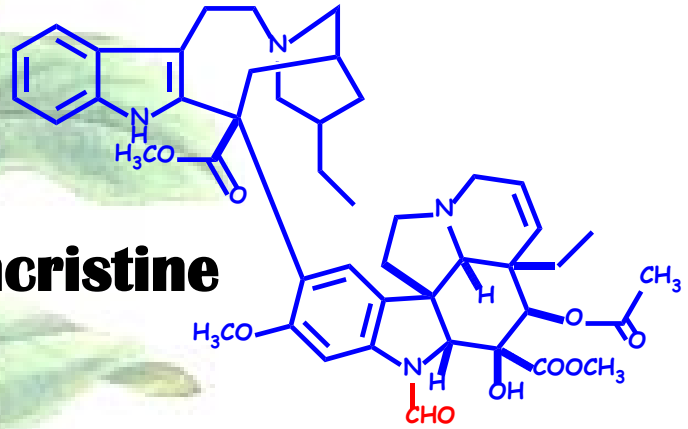
3-,4- anhydro vinblastine

Hydratase



Vinblastine

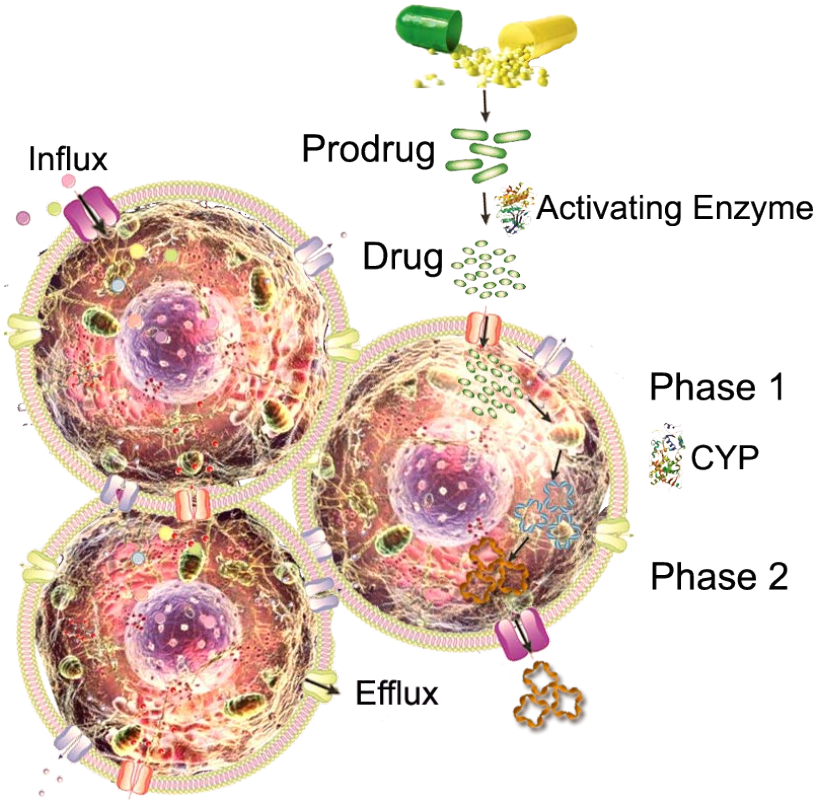
Oxidase

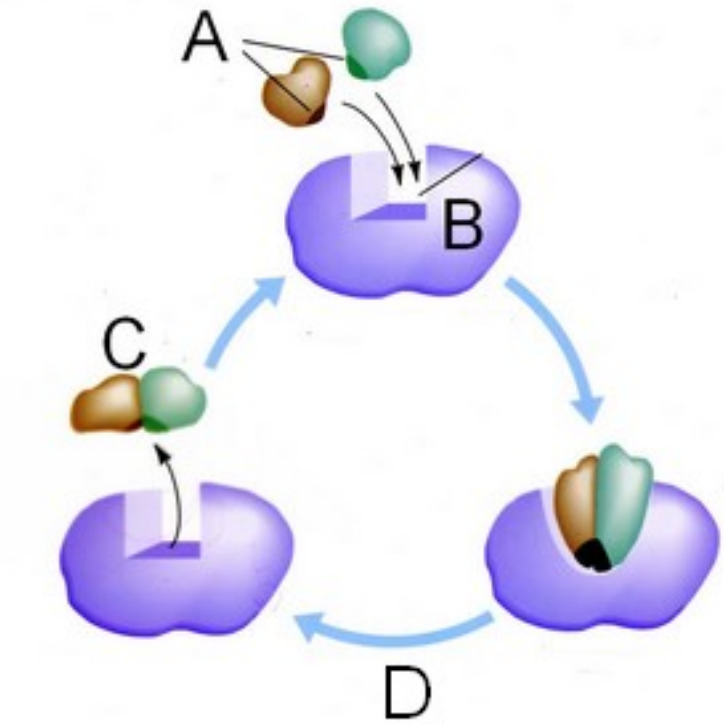


Vincristine

BIOTRANSFORMATION

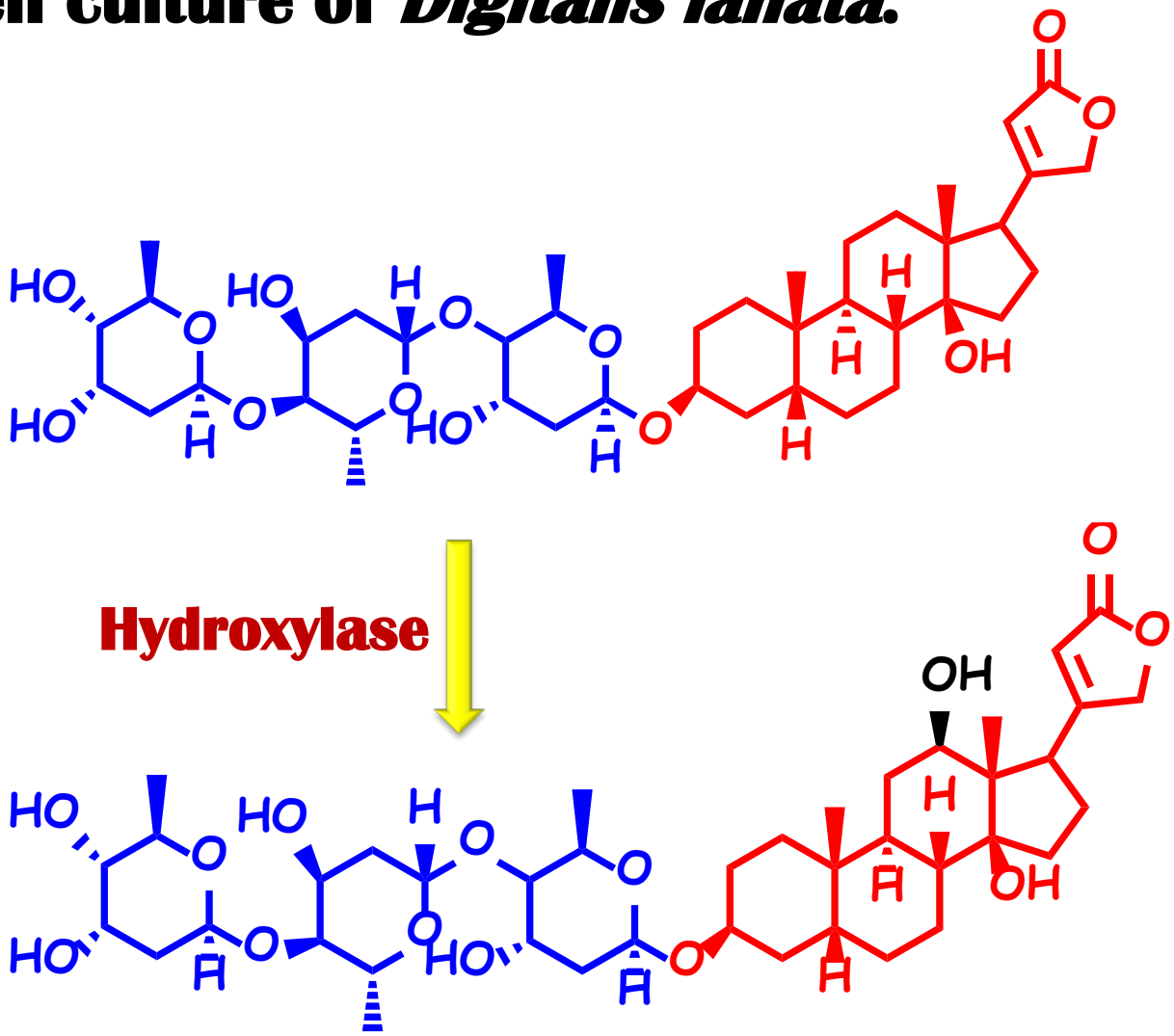
- **Using enzyme to transform less active compound to more active product. e.g. hydroxylation or glycosidation.**





BIOTRANSFORMATION

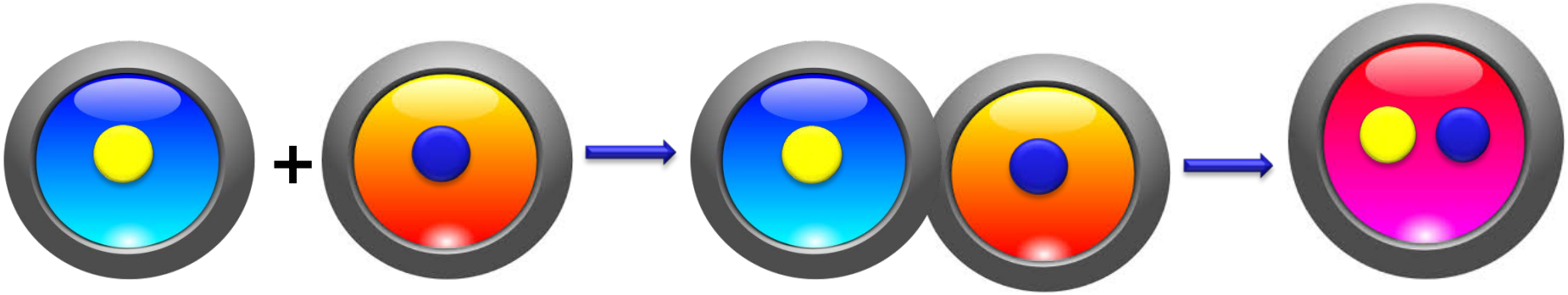
- **Biotransformation of Digitoxin to digoxin by enzyme located in the cell culture of *Digitalis lanata*.**



IMPROVEMENT OF PLANTS BY SOMATIC HYBRIDIZATION

Plant protoplasts

- **Ability of these Plant protoplasts to fuse with each other irrespective of their origin (Protoplast Fusion).**

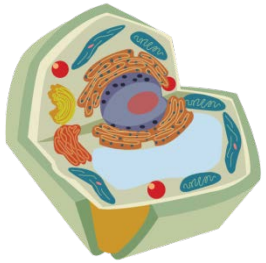


Spontaneous fusion. **Induced fusion.**

SOMATIC HYBRIDIZATION

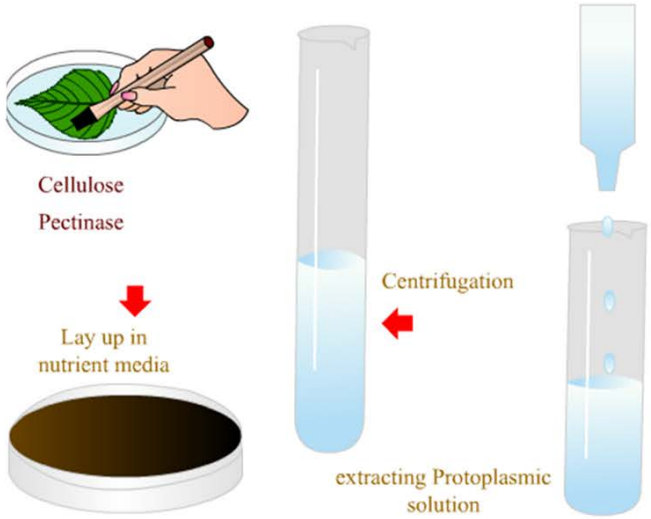
1- ISOLATION OF PROTOPLAST.

Cell Wall



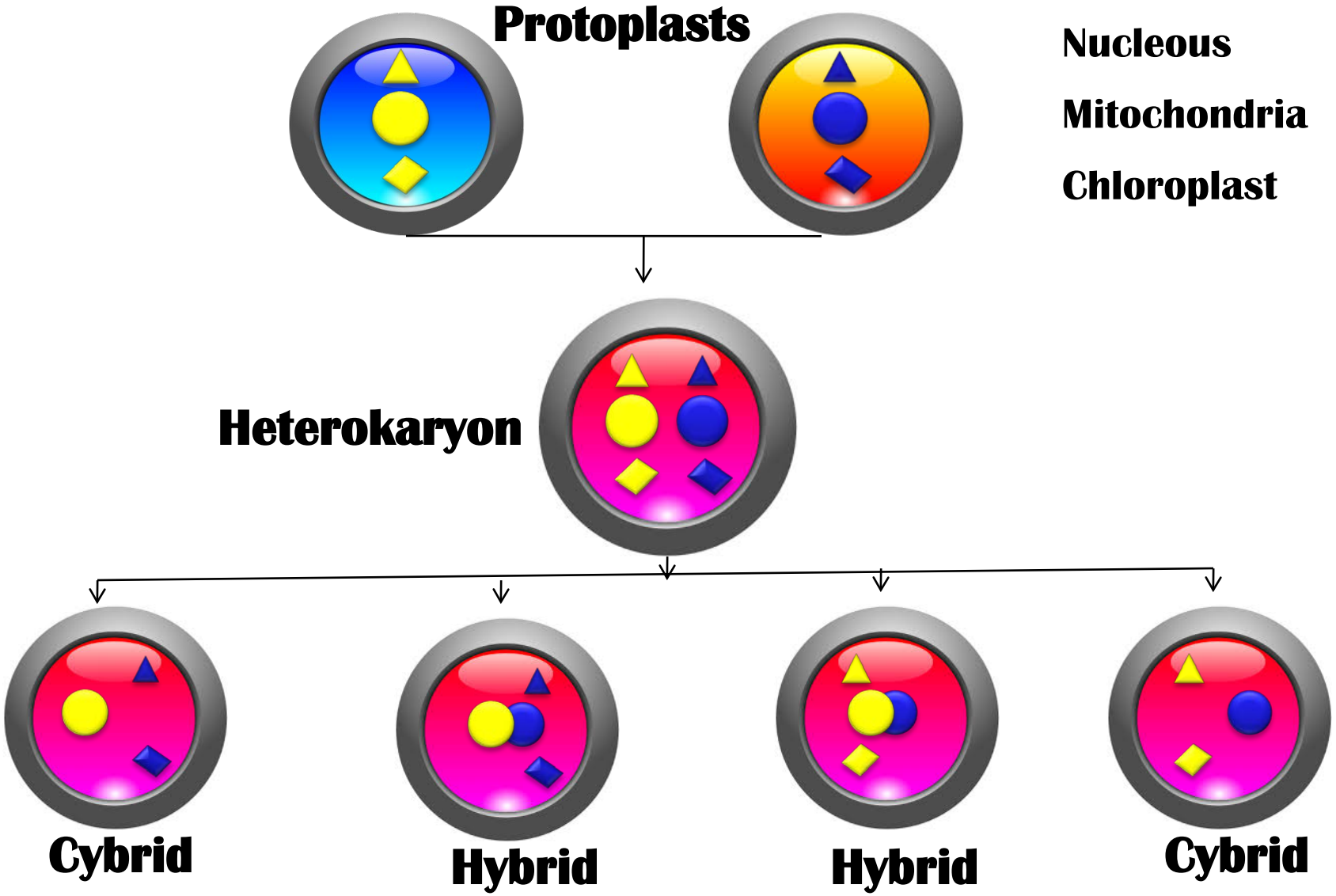
a- Mechanically using a fine knife.

b- The enzymatic isolation (Cellulase - pectinase).



Sterile Leaf + Knife

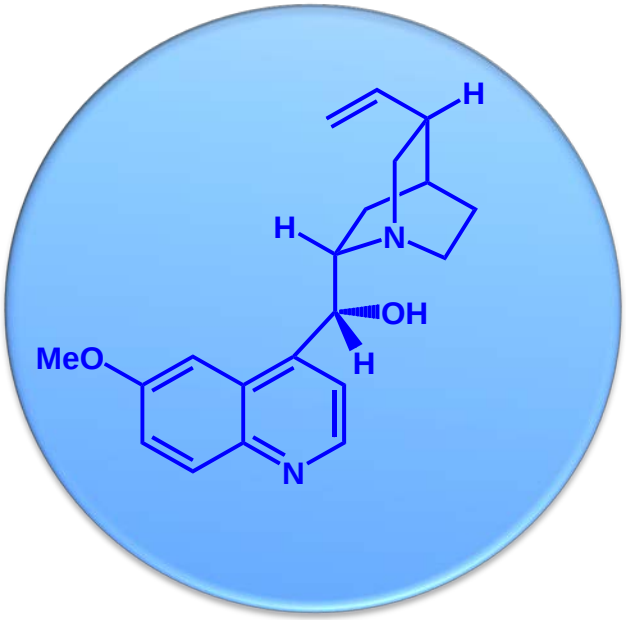
HYBRIDS & CYBRIDS



SOMATIC HYBERDIZATION

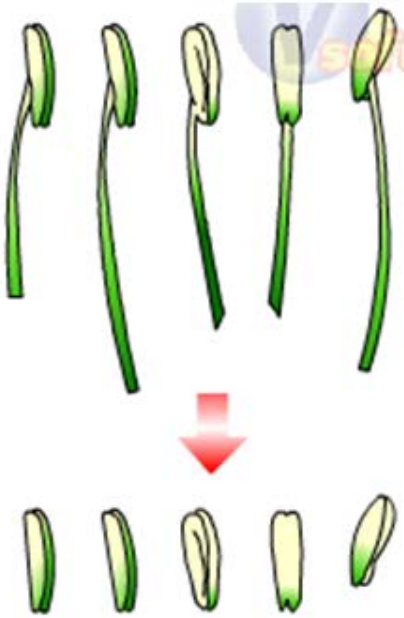
- **Valuable with species which are closely related.**

- ***Cinchona succirubra*** **(3% alkaloids) and** ***Cinchona ledgeriana*** **(5% alkaloids) form a hybrid (11 % alkaloids).**



HAPLOID PLANTS

Selecting the anthers



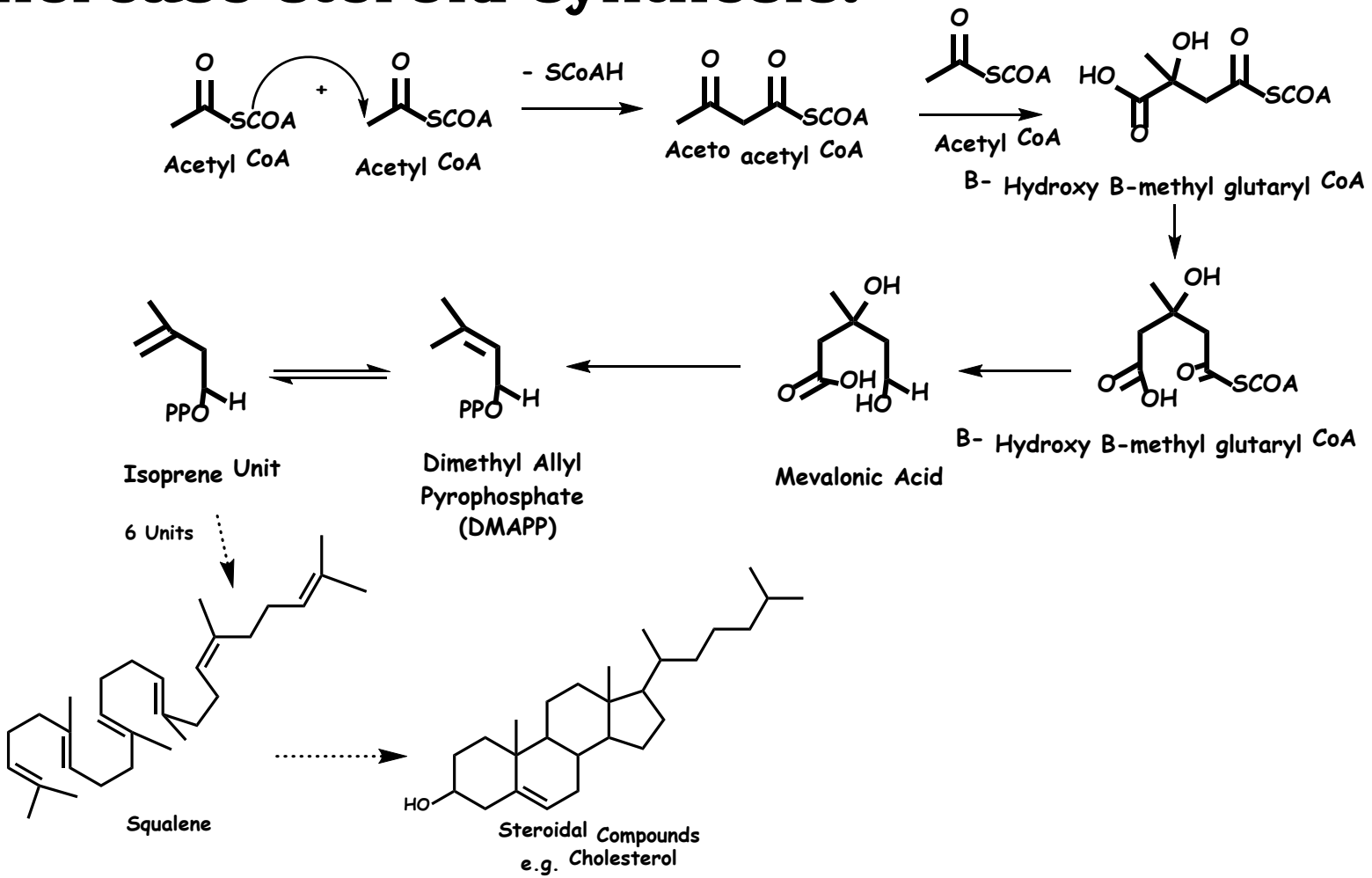
Remove the filament

Culture in solid media

Culture in liquid media

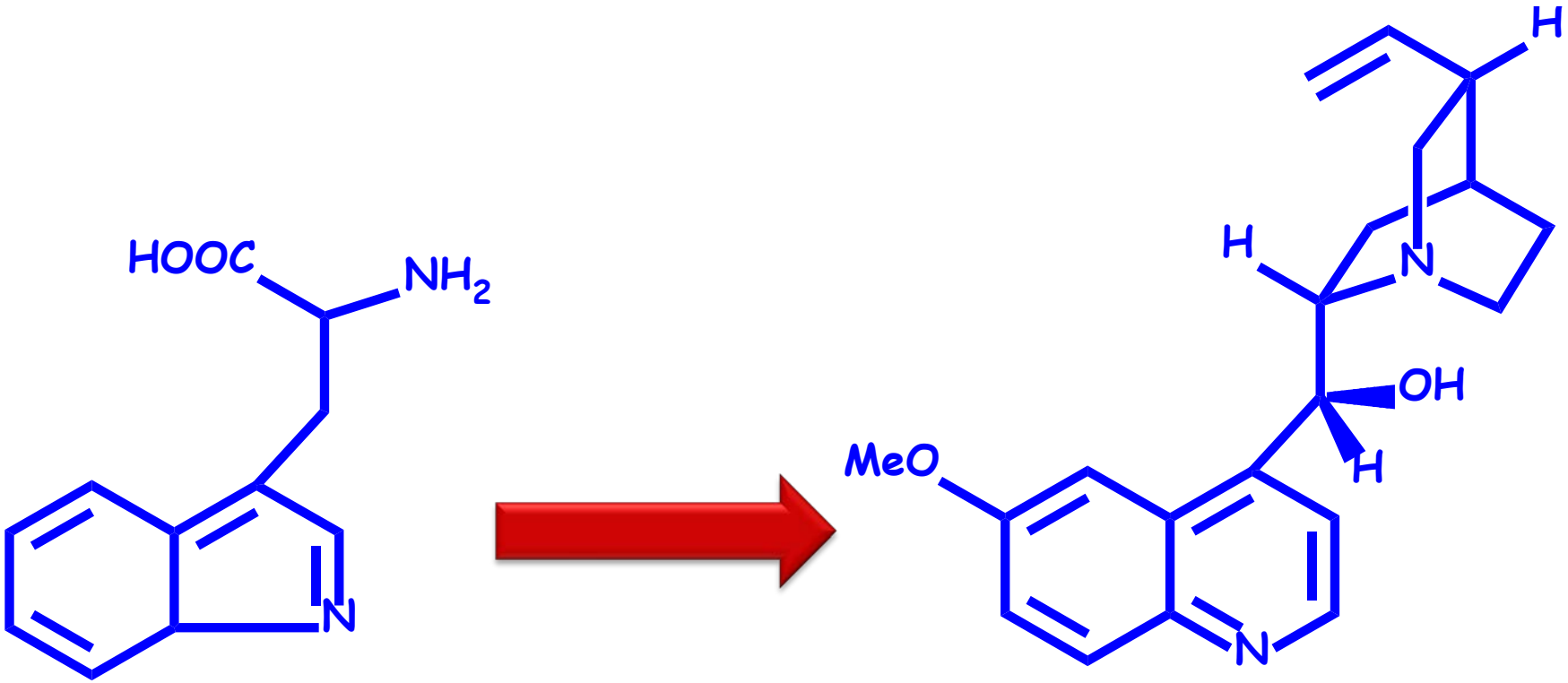
SECONDARY METABOLITES

- **Addition of precursors e.g. Mevalonic acid increase steroid synthesis.**



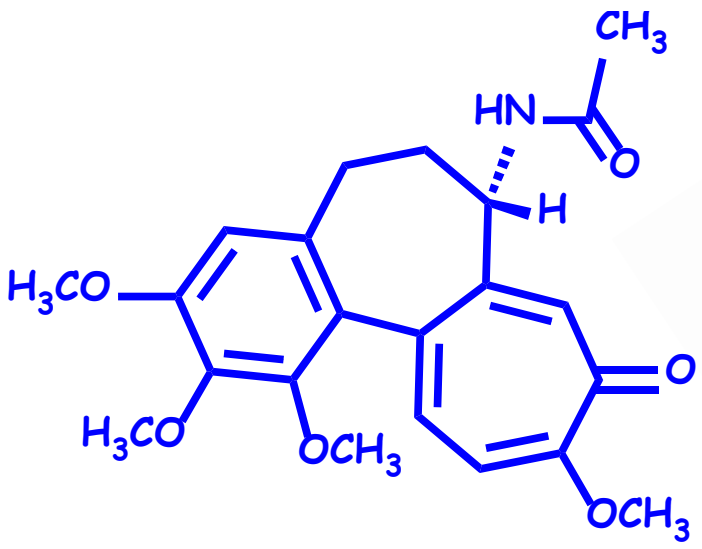
SECONDARY METABOLITES

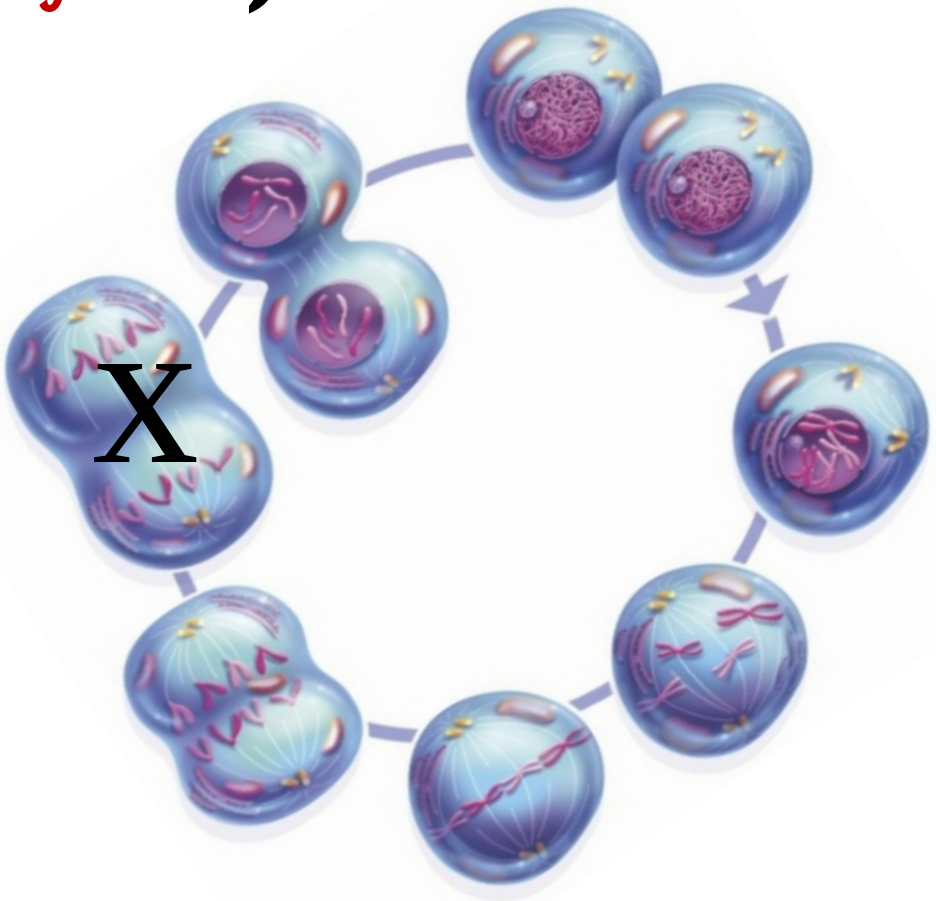
- **Addition of precursor (L-tryptophan) to the culture media of *Cinchona ledegriana* increase its quinoline alkaloid content.**



SECONDARY METABOLITES

- Addition of **colchicine** to cell suspension culture of ***Valeriana spp.*** increase the **valeprotriates** (**sixty fold**).





THANK YOU